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Effects of gestational plane of nutrition and selenium supplementation on mammary development and colostrum quality in pregnant ewe lambs[1]

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ABSTRACT: To examine effects of nutritional plane and Se supplementation on colostrum quality and mammary development, individually fed, pregnant Rambouillet ewe lambs were allotted randomly to 1 of 6 treatments in a 2 × 3 factorial arrangement. Main effects included dietary Se level, which began at breeding (d = 0) [adequate Se (9.5 μg/kg of BW) vs. high Se (81.8 μg/kg of BW)], and plane of nutrition, which began at d 50 of gestation [60% (RES), 100% (CON), and 140% (HIGH) of requirements]. Upon parturition, lambs were immediately separated from dams and weighed. Three hours after lambing, colostrum yield was determined, and samples were obtained for components and immunoglobulin G (IgG) analysis. Ewes were slaughtered within 24 h of parturition, and mammary tissues were collected for determination of alveolar secretory epithelial cell proliferation index and luminal area. Gestation length was reduced ($P < 0.01$) in HIGH ewes compared with RES and CON ewes. Although birth weights were reduced ($P < 0.01$) in RES and HIGH compared with CON ewes, there was little effect of diet on placental size. Mammary gland weight was reduced ($P \leq 0.05$) in RES compared with CON and HIGH, which were similar. However, when expressed as grams per kilogram of BW, mammary gland weight in HIGH ewes was less ($P = 0.03$) compared with RES and CON. Colostrum weight and volume were reduced ($P < 0.01$) in RES and HIGH ewes compared with CON. Although colostrum IgG concentration was greater in RES ewes compared with CON and HIGH, total IgG was lower ($P \leq 0.06$) in RES and HIGH compared with CON ewes. The percentage of alveolar cells proliferating was increased ($P < 0.04$) in HIGH compared with RES ewes, with CON being intermediate. Percentage of alveoli luminal area per unit tissue area was increased ($P = 0.04$) in RES compared with HIGH and CON ewes, which did not differ. Selenium had no effect ($P \geq 0.15$) on mammary gland weight, colostrum quantity, or IgG concentration in pregnant ewe lambs. Improper nutrition from mid to late pregnancy in ewe lambs altered colostrum quality and quantity and reduced offspring birth weight, which may have negative implications for lamb health and survival during the early postnatal period.

Key words: colostrum, ewe lamb, nutritional level, pregnancy, selenium

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INTRODUCTION

Maternal nutrition directly influences milk quantity and quality available to offspring (Miranda et al., 1983). Colostrum provides offspring with immunoglobulins (**Ig**) for passive immunity needed for infection resistance and overall survival (Khalaf et al., 1979). Interestingly, total IgG content in the colostrum is reduced when ewe lambs are overnourished compared with moderately fed controls (Wallace et al., 2006). Immunoglobulin G concentration is increased in colostrum when beef cows were supplemented with Se during late gestation (Awadeh et al., 1998). Moreover, maternal Se supplementation increased colostrum and

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Table 1. Ingredient composition (% of DM) of the treatment pellets that were pen-fed from the end of breeding to d 29 to 36 of pregnancy[1]

Ingredient	ASe (<0.5 mg of Se/kg of DM)	HSe (60 mg of Se/kg of DM)
Wheat midds	72.1	78.8
Selenosource AF 2000[2]	—	3.0
Yeast culture	3.0	—
Sunflower meal	2.8	—
Barley grain	5.0	5.0
Corn grain	8.9	5.0
Alfalfa hay	5.0	5.0
Cane molasses	3.0	3.0
Binding agent	0.2	0.2

[1]ASe = adequate Se; HSe = high Se.
[2]Se concentration = 2,000 mg/kg (DM basis); Diamond V, Cedar Rapids, IA.

liver Se concentrations in newborn calves (Abdelrahman and Kincaid, 1995).

Changing maternal-nutritional status altered mammary gland growth (Mahan, 1990). For example, increased dietary energy enhanced mammogenesis in ewe lambs (McFadden et al., 1990). Energy and protein intake during lactation in sows influenced mammary gland growth, milk yield, and DNA quantity in the glands (Kim et al., 1999). Further, obesity produced abnormal mammary gland morphology in mice, which may have affected milk quality and quantity (Flint et al., 2005).

We hypothesized that maternal under- and overnutrition during pregnancy will negatively affect mammary gland development and colostrum yield in pregnant ewe lambs. Further, we hypothesized that supranutritional Se levels will increase colostrum IgG content. Therefore, objectives were to determine how maternal nutrition and Se supplementation during pregnancy influence mammary tissue, colostrum IgG, and colostrum components.

MATERIALS AND METHODS

Animals and Diets

Animal use and care was approved by the Institutional Animal Care and Use Committees at North Dakota State University, Fargo, and the USDA, ARS, US Sheep Experiment Station in Dubois, Idaho.

At the US Sheep Experiment Station, 160 Rambouillet ewe lambs (age = 240 ± 17 d) were equally divided into 2 breeding groups. Within each breeding group, ewes were divided among 8 pens (n = 10 or 11/pen), estrus was synchronized in all ewes, and a single Rambouillet ram was placed in each pen of ewes for 72 h (1 ram/pen). Marking paint was placed on the briskets of the rams to facilitate identification of ewes that the rams attempted to breed. Subsequently, marked ewes were randomly assigned a treatment pen (n = 2), and Se treatments were randomly assigned to the pens. Selenium treatments were adequate Se (ASe; 9.5 μg/kg of BW) vs. high Se (HSe; 81.8 μg/kg of BW) and were delivered in pellet (Table 1) form as a daily top dressing (100 g/ewe). In our laboratory, Se fed at similar concentrations affected maternal tissue growth in pregnant ewe lambs (Reed et al., 2007). During breeding and 36 and 29 d after breeding, depending on breed group, ewes were fed (2.04 kg/ewe daily) a diet consisting of 47% alfalfa hay, 20% corn, 20% sugar beet pulp pellets, 8% malt barley straw, and 5% concentrated separator by-product (DM basis). For breeding groups 1 and 2, pregnancy was determined 36 and 29 d after breeding, respectively. Eighty-two pregnant ewes were identified and shipped (1,544 km; ~14 h) to the Animal Nutrition and Physiology Center at North Dakota State University for the remainder of the experiment. From breeding groups 1 and 2, forty-five and 37 ewes were selected, respectively. Ultimately, 40 and 42 ewes remained in the ASe and HSe treatments groups, respectively.

Upon arrival at North Dakota State University, ewes remained on their assigned Se treatments. Ewes were individually housed in 0.91 × 1.2-m pens in a temperature controlled (12°C) and ventilated facility for the duration of the study. Lighting within the facility was automatically timed to mimic daylight patterns. On d 50 of gestation, ewes were assigned randomly to 1 of 3 nutritional diets: 60% (RES), 100% (CON), or 140% (HIGH) of the NRC (1985) requirements, resulting in a randomized design with a 2 × 3 factorial arrangement of treatments [ASe-RES (n = 14); ASe-CON (n = 13); ASe-HIGH (n = 13); HSe-RES (n = 14); HSe-CON (n = 14); HSe-HIGH (n = 14)]. All diets were fed once daily in a complete pelleted form (0.48-cm diameter; Table 2). Ewes had free access to water and a trace mineralized salt block [containing no added Se; maximum NaCl (99%), minimum NaCl (96%), and verified minimum amounts of the following: Mn (2,000 mg/kg), Fe (1,000 mg/kg), Mg (1,000 mg/kg), S (500 mg/kg), Cu (250 mg/kg), Co (100 mg/kg), Zn (80 mg/kg), and I (70 mg/kg); Roto Salt Company, Penn Yan, NY]. Nutrient requirements were based on NRC (1985) recommendations for 60-kg BW, pregnant ewe lambs during mid to late gestation (weighted ADG of 140 g/d). Intake of the respective diets was calculated based on BW, ME requirements, and supplement ME and Se concentrations. Body weight was measured every 14 d, and diets were adjusted accordingly.

Colostrum Collection and Analyses

All births were observed. Upon parturition, lambs were immediately separated from their dams, and the lambs were weighed. The placenta was weighed upon delivery. Thereafter, cotyledons were dissected, counted, and a total cotyledonary weight was recorded. Exactly 3 h after parturition, ewes were injected with 1 mL of oxytocin (20 IU; AgriLabs, St. Joseph, MO) i.v. to facilitate colostrum ejection, and colostrum was manu-

ally collected into clean containers. Colostrum yield was measured (g and mL) and frozen at −20°C for IgG and Se analysis. Further, an aliquot (20 to 30 mL) of colostrum was mixed with Broad Spectrum Microtabs II (D & F Control Systems, Dublin, CA) and shipped to Heart of America DHIA (Manhattan, KS) for analysis of butterfat, protein, somatic cell count (**SCC**), lactose, solids not fat, and milk urea N (**MUN**). Colostral IgG concentrations were determined by radial immunodiffusion using a commercially available kit (Bethyl Laboratories Inc., Montgomery, TX). All samples and controls were run in triplicate. The interassay CV of the high, medium, and low pool controls averaged 5.1, 6.3, and 2.8%, respectively. Further, the intraassay CV was 9.6%. Sensitivity of the assay was 125 mg/dL.

Slaughter Procedures and Mammary Gland Processing

Ewes were slaughtered between 3 and 22 h after parturition. Before slaughter, each ewe was weighed to obtain a final BW. A blood sample was obtained from each ewe lamb via jugular venipuncture, and thereafter, 1 mL of oxytocin (20 IU; AgriLabs) was delivered to facilitate milk ejection. Milk was manually collected immediately before slaughter to obtain an empty mammary gland weight. Ewes were stunned by captive bolt (Supercash Mark 2, Acceles and Shelvoke Ltd., Birmingham, UK), exsanguinated, and maternal tissues were harvested. The digesta was stripped from the gastrointestinal tract. The entire mammary gland was dissected from the skin, weighed, and immediately processed.

From one-half of the gland, 5 samples (approximately 1 g each) of mammary gland were snap-frozen in super-cooled isopentane (submerged in liquid nitrogen) and stored at −80°C until analysis for RNA, DNA, and protein (Reynolds et al., 1990; Reynolds and Redmer, 1992). The remaining half of the mammary gland was immediately perfusion-fixed with Carnoy's fixative (70% ethanol, 30% acetic acid, 10% chloroform) by cannulating the cranial mammary artery with a polyethylene (PE-60; o.d. = 1.22 mm; i.d. = 0.77 mm; Intramedic, Becton Dickinson & Company, Sparks, MD) beveled catheter that was secured to surrounding tissue. The mammary gland was initially perfused with PBS, then with Evan's blue dye (to define the vasculature), then with PBS again, and then, finally, was perfusion-fixed with Carnoy's fixative. Mammary tissue was then cut into ~1-cm cubes and was further immersion-fixed in Carnoy's fixative for an additional 24 h. Thereafter, mammary gland tissues were dehydrated in a series of ethanol, Histo-Clear (National Diagnostics, Atlanta, GA), and embedded in paraffin wax.

Cellular Proliferation Index

The paraffin-embedded tissues were sectioned at 5 μm and stained for a cellular proliferation marker using the mouse antiproliferating nuclear cell antigen primary antibody (clone PC-10; Chemicon International, Temecula, CA) and detected with a secondary biotinylated secondary antibody (horse anti-mouse IgG; Vectastain, Vector Laboratories, Burlingame, CA) and the Avidin-Biotin Complex system (Vectastain, Vector Laboratories). Tissues were further stained with periodic acid-Schiff's reagent and counterstained with hematoxylin. Photomicrographs of alveoli were taken with 400× magnification using a Nikon Eclipse E800 microscope (Nikon Instruments Inc., Melville, NY). Five images were randomly taken throughout the tissue section for each ewe and were analyzed for proliferating alveolar cells and alveolar luminal area using the Image-Pro Plus image analysis software package (Image-Pro Plus version 5.0, Media Cybernetics, Houston, TX), as we have previously reported for intestinal (Reed et al., 2007; Neville et al., 2008) and placental tissues (Borowicz et al., 2007; Vonnahme et al., 2007b).

Table 2. Diet composition and calculated nutrient composition of diets fed to gestating ewe lambs (DM basis) while at North Dakota State University

Item	Basal pellet	Selenium pellet
Ingredient, % of dietary DM		
Beet pulp, dehydrated	36.5	36.5
Alfalfa meal, dehydrated	22.3	22.3
Ground corn	16.2	16.2
Soybean hulls	18.0	18.0
Soybean meal	7.0	4.9
Selenium-enriched yeast[1]	—	2.1
Analyzed dietary composition, % DM		
OM	93.8	93.8
CP	14.4	14.1
NDF	38.9	39.5
ADF	25.4	25.7
Starch	13.2	12.9
Ca	0.93	0.84
P	0.24	0.24
Se, mg/kg	0.77	40.4
Calculated dietary composition, Mcal/kg		
Metabolizable energy[2]	2.63	2.65

[1]Diamond V Mills Inc., Cedar Rapids, IA; 2,000 mg/kg of Se.
[2]Estimated using values obtained from the NRC (1985).

Cellularity Estimates

Freshly thawed tissue samples were homogenized using a Polytron with PT-10s probe (Brinkmann, Westbury, NY) in Tris aminomethane, sodium, and EDTA buffer (TNE buffer; 0.05 *M* Tris, 2.0 *M* NaCl, 2 m*M* EDTA, pH 7.4). Samples were then analyzed for concentrations of DNA and RNA, as we have reported before, by using the diphenylamine (Johnson et al., 1997) and orcinol procedures (Reynolds et al., 1990). Protein in tissue homogenates was determined with Coomassie Brilliant Blue G (Bradford, 1976), with bovine serum albumin (Fraction V, Sigma Chemical, St. Louis, MO) as the standard (Johnson et al., 1997). Prepared samples

were analyzed with a spectrophotometer (Beckman DU 640, Beckman Coulter Inc., Fullerton, CA) and were assessed against concentration curves of known standards. Concentration of DNA was used as an index of hyperplasia, and the protein:DNA and RNA:DNA ratios were used as indices of hypertrophy and potential cellular activity (Swanson et al., 2000; Scheaffer et al., 2003; Soto-Navarro et al., 2004).

Dietary Analysis

Diet samples were analyzed for DM, ash, N (methods 930.15, 942.05, and 990.02, respectively; AOAC, 1990), ADF, and NDF (Ankom, Fairport, NY). Diet, colostrum, and plasma samples were prepared for Se analysis. Hydride generation atomic absorption spectroscopy (5100 AAS, Perkin-Elmer Inc., Boston, MA) was used for Se analysis, as previously reported (Finley et al., 1996).

Calculations

Empty BW (**EBW**) was calculated as BW minus total digesta weight. To express mammary mass on an EBW basis, fresh organ mass (g) was divided by EBW (kg). The mammary gland weight:fetal weight ratio was calculated to determine if there was a difference in nutrient partitioning during gestation as described previously by Mellor (1987). Percentage of alveolar proliferating cells was calculated by dividing the number of proliferating nuclear cell antigen-positively stained cells by the total number of cells in the alveolar section, multiplied by 100. Furthermore, to determine the alveolar area per unit of tissue area, total alveolar luminal area per slide was divided by total tissue area per slide and multiplied by 100 to determine the percentage of alveolar area per tissue area. Total DNA, RNA, and protein contents were calculated by multiplying the DNA, RNA, and protein concentration by fresh tissue weight (Swanson et al., 2000; Scheaffer et al., 2003, 2004).

Statistics

Data were analyzed as a completely randomized design with a 2 × 3 factorial arrangement of treatments using an ANOVA (PROC GLM; SAS Inst. Inc., Cary, NC). Because ewes carried singles (n = 70) and twins (n = 8), fetal number was included in the model as a covariate. If fetal number was significant ($P < 0.10$), it was retained in the model. The model contained level of Se (ASe vs. HSe), nutritional level (RES, CON, HIGH), and the Se × nutritional level interaction. When interactions were present ($P < 0.10$), means were separated by LSD test.

RESULTS

Four ewes were removed from the study, because 3 (ASe-CON, ASe-HIGH, HSe-CON) were not pregnant due to loss of pregnancy, and 1 (ASe-RES) did not adapt to the diet. Immediately before nutritional treatment implementation, initial BW was similar ($P = 0.93$ and 0.23 for nutrition and Se, respectively; Table 3). Postpartum final BW, carcass weight, and EBW in RES ewes were lighter ($P \leq 0.01$) than CON and HIGH, and CON were lighter than ($P \leq 0.01$) HIGH ewes (Table 3). Nutritional levels did not affect plasma Se concentrations, whereas HSe ewes had increased ($P < 0.01$) plasma Se concentrations immediately postpartum compared with ASe ewes (0.97 ± 0.07 vs. 0.22 ± 0.01 mg/kg).

Gestation length was decreased ($P < 0.01$) in HIGH compared with RES and CON ewes, which did not differ (Table 3). Birth weights were reduced ($P < 0.01$) in RES and HIGH ewes compared with the CON, and birth weights from RES and HIGH ewes were not different. There was no effect of diet on placental weight, cotyledonary number, or cotyledonary weight. However, HIGH ewes had a lighter ($P = 0.04$) cotyledonary weight compared with RES in an unprotected LSD. There was no effect ($P \geq 0.16$) of Se on gestation length, birth weight, or placental weight measurements (Table 3).

Mammary gland weight (g) was lighter ($P \leq 0.05$) in RES compared with CON and HIGH, which did not differ. However, when expressed as grams per kilogram of EBW, mammary gland weight in HIGH ewes was reduced ($P = 0.03$) compared with RES and CON ewes, which did not differ. Colostrum weight and colostrum volume were reduced ($P < 0.01$) in RES and HIGH compared with CON ewes. Although colostrum IgG concentration was greater in RES ewes compared with CON and HIGH, total IgG production (g) was less ($P \leq 0.06$) in RES and HIGH compared with CON. Selenium had no effect ($P \geq 0.15$) on maternal BW, mammary gland weight, colostrum quantity, or IgG concentration in pregnant ewes. There was no effect of treatments ($P \geq 0.54$) on the mammary gland:birth weight ratio, which averaged 0.17 ± 0.01.

There was a Se × nutritional level interaction ($P < 0.01$) on colostrum Se concentration. Nutritional level had no effect on Se concentration in colostrum from ASe ewes, whereas HSe-RES ewes had increased ($P < 0.01$) Se concentration compared with HSe-CON and HSe-HIGH ewes, which did not differ (Figure 1A). When total colostrum Se content was calculated, HSe ewes had increased ($P < 0.01$) Se content (μg) compared with ASe. Further, there was a nutritional level effect with HIGH ewes having reduced total colostrum Se content compared with CON, with RES ewes being intermediate (Figure 1B).

There were no interactions of Se × nutrition ($P \geq 0.17$) or main effects ($P \geq 0.42$) of Se on DNA, RNA, and protein in mammary tissue (Table 4). Although nutrition did not affect mammary gland DNA, RNA, and protein concentrations, protein content, or RNA:DNA and protein:DNA ratios, total DNA, and RNA contents were reduced ($P \leq 0.03$) in mammary glands from RES

Table 3. Effect of selenium and nutritional level on maternal BW, gestation length, lamb birth weight, placental weight, mammary gland weight, colostrum weight and volume, and immunoglobulin G (IgG) concentration and content in pregnant ewe lambs

	Nutrition treatment[1]				Selenium treatment[2]			*P*-value[3]		
Item	RES	CON	HIGH	SE	ASe	HSe	SE	Nut	Se	Nut × Se
Initial BW, kg	52.1	52.3	51.8	0.9	52.7	51.4	0.8	0.93	0.23	0.87
Final BW, kg	48.1[a]	58.5[b]	69.1[c]	1.1	59.1	58.0	0.9	0.01	0.39	0.85
Gestation length, d	150.0[a]	149.8[a]	146.9[b]	0.4	149.3	148.5	0.4	<0.01	0.16	0.88
Empty carcass,[4] kg	32.4[a]	40.1[b]	49.3[c]	0.9	41.3	39.9	0.7	0.01	0.16	0.44
EBW,[5] kg	43.2[a]	52.8[b]	63.8[c]	1.0	53.7	52.8	0.8	<0.01	0.43	0.84
Lamb birth wt, kg	4.01[a]	4.64[b]	4.21[a]	0.13	4.36	4.22	0.10	<0.01	0.33	0.59
Placenta wt, g	363.2	389.7	339.2	19.7	364.8	363.3	15.7	0.20	0.95	0.89
Cotyledonary number	86.9	90.0	87.6	3.8	85.5	90.8	3.0	0.82	0.22	0.75
Cotyledon wt, g	116.9	110.2	93.4	8.7	108.6	105.1	6.7	0.12[6]	0.72	0.98
Mammary gland, g	670.1[a]	838.9[b]	815.3[b]	48.5	735.7	813.8	39.9	0.03	0.16	0.96
g/kg of EBW[7]	15.7[a]	16.0[a]	12.9[b]	1.0	14.0	15.7	0.8	0.05	0.15	0.98
Colostrum weight, g	343.8[a]	585.7[b]	364.0[a]	50.9	437.0	425.3	41.9	<0.01	0.84	0.93
g/kg of EBW[7]	8.04[a]	11.42[b]	5.68[a]	1.08	8.53	8.23	0.89	<0.01	0.80	0.92
Colostrum volume, mL	325.9[a]	575.1[b]	364.0[a]	51.3	436.6	406.7	42.7	<0.01	0.61	0.93
Colostrum Se, μg/g	2.11	1.75	1.63	0.10	0.45	3.21	0.09	<0.01	<0.01	<0.01
Total Se, μg	713.7[a]	1,033.3[b]	528.4[b]	139.2	177.2	1,339.7	114.7	0.04	<0.01	0.31
Colostrum IgG, g/L	127.7[a]	82.1[b]	99.9[b]	8.3	96.9	109.6	6.8	<0.01	0.18	0.35
Total IgG, g	31.5[a]	43.2[b]	33.6[a]	3.6	36.2	36.1	3.0	0.06	0.98	0.52

[a–c]Within a row, means for nutritional treatment differ ($P \leq 0.06$).

[1]Nutritional treatments were RES (60% of control), CON (control; 100% requirements for gestating ewe lambs), and HIGH (140% of control).

[2]Selenium treatments were daily intake of organically bound Se, adequate Se (ASe; 9.5 μg/kg of BW) vs. high Se (HSe; 81.8 μg/kg of BW).

[3]Probability values for effects of nutrition (Nut), selenium (Se), and the interaction.

[4]Empty carcass = carcass (head, hide, and carcass) − total internal organs.

[5]Empty BW (EBW) = final BW − digesta weight.

[6]Unprotected LSD; $P = 0.04$ RES > HIGH.

[7]g/kg of EBW = tissue mass (g)/EBW (kg).

compared with CON ewes, which did not differ from HIGH ewes. The percentage of alveolar cells proliferating was increased ($P < 0.04$) in HIGH ewes compared with RES, with CON ewes being intermediate. Furthermore, the percentage of alveoli luminal area per unit tissue area was increased ($P = 0.04$) in RES compared with HIGH and CON ewes, which did not differ.

Density of colostrum (g/mL) was increased ($P = 0.05$) in RES versus CON, with colostrum from HIGH ewes being intermediate (Table 5). The RES and HIGH ewes had decreased ($P \leq 0.02$) total amounts (g) of butterfat, protein, lactose, and solids not fat components compared with colostrum from CON. Expressed as a concentration, MUN increased ($P < 0.01$) as nutritional level increased (Table 5). Total amount of MUN was reduced in colostrum from RES compared with CON or HIGH ewes, which did not differ (Table 5). Although Se treatment alone had no effect on any colostrum component, a Se × nutrition interaction in percentage protein and solids not fat in colostrum was observed ($P = 0.03$, Figure 2). In ASe ewes, nutritional level did not affect protein or solids not fat percentage. In HSe ewes, HIGH ewes had an increased ($P = 0.03$) protein and solids not fat percentage compared with RES and CON, which did not differ. Further, ASe-RES ewes had a greater colostral percentage of protein and solids not fat compared with HSe-RES (Figure 2). There was no effect of nutritional level or Se on concentration or total SCC in colostrum.

DISCUSSION

To our knowledge, these are the first data to directly compare the combined effects of both under- and overnutrition and maternal Se intake from mid to late gestation on mammary gland growth and colostrum composition in first-parity ewes. In our study, although there was little effect of Se on mammary growth, modifications in nutritional level during the last two-thirds of pregnancy greatly affected birth weight, the mammary gland, and colostrum components.

Although mammary gland weight was reduced in RES compared with CON and HIGH ewes, the proportional size of mammary gland was only reduced in the HIGH ewes. The reduction in absolute or proportional size reduced the amount of colostrum produced. In agreement, reduced colostrum yield has been previously reported for underfed ewes (Mellor and Murray, 1985) and overfed primiparous ewes (Wallace et al., 2005, 2006). It is interesting to note that a more severe nutrient restriction (50% maintenance; Mellor and Murray, 1985) or excess (i.e., 200% maintenance; Wallace et al., 2005) have both reported similar results.

Therefore, colostrum quality and quantity appear to be regulated by maternal nutrition.

Similar to other reproductive tissues, the mammary gland grows allometrically during pregnancy. In ewes, 98% of mammary gland growth occurs during pregnancy, where the differentiation and growth of alveolar epithelial cells are maximal during the latter stages of gestation and the remaining 2% of growth occurs during lactation (Anderson, 1975). Therefore, there is competition between the gland and the growing conceptus for nutrients during late pregnancy. Decreases in nutrient intake during late pregnancy have been shown to markedly reduce the udder measurements and colostrum yields in ewes (Mellor and Murray, 1985). During the last 4 wk of gestation, it is estimated that 70% of ovine mammary gland growth and 43% of fetal growth are occurring (Koong et al., 1975; Mellor and Murray, 1985; Robinson, 1986). Therefore, competition for nutrients between the mammary gland and fetus may hinder one, or both, of their growth rates. In this study, when mammary gland weight:total lamb birth weight ratio was calculated, there was no effect of Se or nutritional level indicating a synchronous growth, or a lack of disproportionate growth, between the tissues.

Gestation length was 2 d shorter in HIGH ewes, which is similar to reports from Wallace et al. (2005). Nutrient restriction from mid to late pregnancy does not seem to decrease gestation length as has been reported in ewes that were restricted 60 d before conception to 30 d after breeding (Bloomfield et al., 2003, 2004). Although the decrease in gestation length most likely influenced some of the reduction in birth weight in the HIGH ewes, reductions in birth weight also occurred in RES with similar gestation lengths as our CON ewes. Several authors have described reduced fetal weights near the end of gestation, or reduced lamb birth weights in models of nutrient restriction from mid to late pregnancy (Luther et al., 2005). Further, overnourishing primiparous ewe lambs from conception to term results in reduced birth weights (Wallace et al., 2002a,b, 2004), albeit more severe than what is reported here. Competition for nutrients between the immature ewe and exponentially growing fetus may further influence the reductions in birth weight.

In this study, estimates of cellularity (i.e., DNA, RNA, protein concentrations) of the mammary gland did not differ unless they were based on mammary gland weight. However, histological analysis suggested that the alveolar proliferation and size changed based on nutritional level, but not Se level. Alveolar epithelium had an increased proliferation index in HIGH compared with RES and CON ewes, indicating that the increased level of nutrients stimulated alveolar growth. Furthermore, increases in the alveolar proliferation index may be indicative of an earlier differentiation of the gland, because preliminary data in our laboratory demonstrates decreased circulating estrogen and progesterone concentrations in HIGH compared with RES and CON ewes (Vonnahme et al., 2007a; our unpublished data). Decreases in estradiol reduce glucocorticoid-binding protein, allowing free cortisol to further complete cellular differentiation in preparation for lactogenesis (Tucker, 1985). Progesterone is needed for lobular alveolar growth in the mammary gland (Tucker, 1985). Beginning on d 64 of gestation, circulating levels of progesterone were increased in RES compared with HIGH and CON ewes (Vonnahme et al., 2007a). This increased duration of greater levels of progesterone may have caused an increase in lobular alveolar development in the RES ewes, resulting in the increased percentage of alveolar luminal area observed in this study. The increased numbers of alveoli did not result in an increase in colostrum production; however,

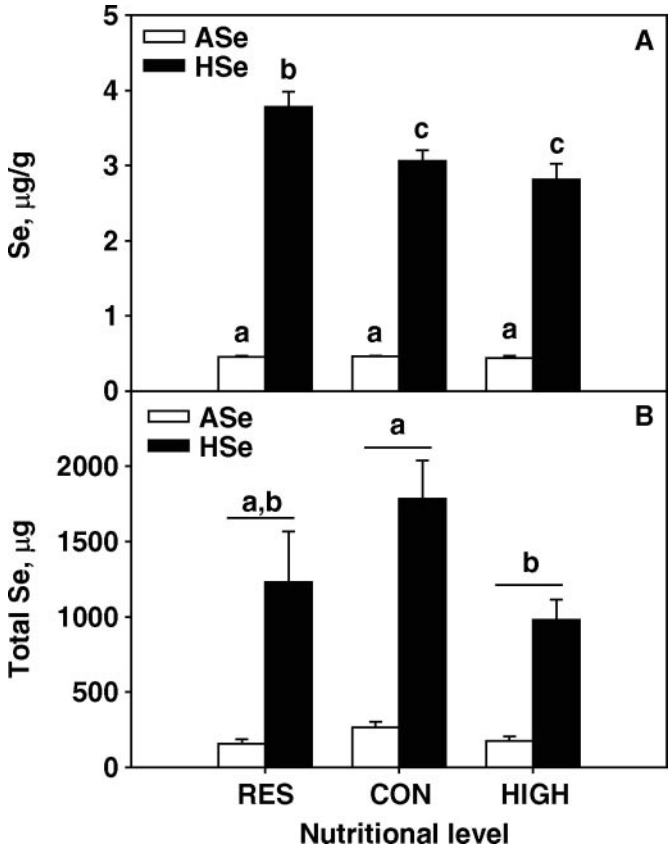

Figure 1. The effects of Se and nutritional level on Se (A) concentration and (B) content in colostrum of pregnant ewe lambs. (A) A nutritional level × Se interaction ($P < 0.01$) was observed for Se concentrations in colostrum; [a–c]means ± SE differ. (B) When total Se content was calculated (Se concentration multiplied by total g of colostrum), there was a nutritional level effect ($P = 0.04$; [a,b]means ± SE differ) and a Se effect ($P < 0.01$), with colostrum from HSe ewes greater than from ASe ewes. Nutritional treatments were RES (60% of control), CON (control; 100% requirements for gestating ewe lambs), and HIGH (140% of control). Selenium treatments were daily intake of organically bound Se, adequate Se (ASe; 9.5 µg/kg of BW) vs. high Se (HSe; 81.8 µg/kg of BW).

Table 4. Effect of selenium and nutritional level on mammary gland DNA, RNA, and protein concentration and content and cellularity proliferation measurements of pregnant ewe lambs

	Nutrition treatment[1]				Selenium treatment[2]			*P*-values[3]		
Item	RES	CON	HIGH	SE	ASe	HSe	SE	Nut	Se	Nut × Se
DNA, mg/g	2.25	2.65	2.39	0.19	2.47	2.39	0.15	0.32	0.73	0.22
DNA, g	1.52[a]	2.18[b]	1.85[ab]	0.18	1.80	1.90	0.14	0.03	0.58	0.39
RNA, mg/g	3.54	4.22	4.01	0.36	4.05	3.79	0.29	0.38	0.51	0.17
RNA, g	2.21[a]	3.42[b]	3.15[b]	0.28	2.90	2.94	0.22	<0.01	0.90	0.25
RNA:DNA	1.65	1.76	1.70	0.16	1.75	1.66	0.13	0.88	0.58	0.58
Protein, mg/g	47.59	44.75	48.71	5.38	45.44	48.60	4.33	0.86	0.60	0.70
Protein, g	29.94	39.05	40.28	4.92	34.19	38.66	3.96	0.24	0.42	0.96
Protein:DNA	25.48	22.77	25.33	4.21	23.00	26.05	3.39	0.87	0.52	0.78
Proliferation,[4] %	6.29[a]	7.76[ab]	9.67[b]	0.95	8.20	7.62	0.78	0.04	0.59	0.43
Alveolar area,[5] %	15.24[a]	11.30[b]	10.46[b]	1.43	12.11	12.56	1.11	0.04	0.78	0.35

[a–c]Within a row, means for nutritional treatment differ ($P < 0.05$)

[1]Nutritional treatments were RES (60% of control), CON (control; 100% requirements for gestating ewe lambs), and HIGH (140% of control).

[2]Selenium treatments were daily intake of organically bound Se, adequate Se (ASe; 9.5 μg/kg of BW) vs. high Se (HSe; 81.8 μg/kg of BW).

[3]Probability values for effects of nutrition (Nut), selenium (Se), and the interaction.

[4]Percentage of proliferation of mammary alveolar cells.

[5]Percentage of the alveolar luminal area per unit of tissue area measured.

milk production over an entire lactation in these ewes has not been investigated.

Nutritional levels not only affected the IgG content in the colostrum in both RES and HIGH ewes but also reduced total butterfat, protein, lactose, and solids not fat in the RES and HIGH ewes compared with CON ewes. Our reductions in butterfat, protein, lactose, solids not fat, and MUN appear to be proportional, because concentrations did not differ among nutritional treatments. However, when considering that total amounts of these essential components during the first days of life are reduced by 30 to 40%, requirements of the neonate would most likely not be met and, without intervention, could lead to hypothermia and neonatal death.

Table 5. Effect of selenium and nutritional level on colostrum components of pregnant ewe lambs

	Nutrition treatment[1]				Selenium treatment[2]			*P*-values[3]		
Item	RES	CON	HIGH	SE	ASe	HSe	SE	Nut	Se	Nut × Se
Colostrum[4]										
Density, g/mL	1.08[a]	1.01[b]	1.04[ab]	0.02	1.03	1.06	0.02	0.05	0.26	0.39
Butterfat,[5] %	13.82	14.45	13.26	0.57	13.81	13.88	0.45	0.29	0.90	0.67
Butterfat,[6] g	52.40[a]	83.96[b]	50.94[a]	8.03	64.42	60.45	6.42	<0.01	0.65	0.94
Protein,[5] %	16.94	16.58	17.69	0.44	17.18	16.97	0.35	0.17	0.66	0.03
Protein,[6] g	66.77[a]	95.97[b]	64.33[a]	9.03	77.64	73.74	7.17	0.02	0.69	0.99
SCC,[5,7] number of cells/mL × 1,000	1,550.85	1,653.26	1,282.42	722.89	1,432.30	1,558.72	577.61	0.92	0.87	0.52
Total SCC,[8] number of cells × 1,000	432,029.27	560,771.94	305,758.32	130,292.36	410,906.23	454,800.13	104,107.43	0.33	0.76	0.93
Lactose,[5] %	2.92	3.12	3.14	0.11	3.12	3.00	0.09	0.31	0.31	0.41
Lactose,[6] g	12.07[a]	18.45[b]	11.97[a]	1.91	14.83	13.49	1.51	0.02	0.52	0.81
Solids not fat,[5] %	23.45	23.19	24.44	0.49	23.89	23.50	0.39	0.13	0.47	0.03
Solids not fat,[6] g	92.80[a]	134.46[b]	89.75[a]	12.69	108.72	102.63	10.08	0.02	0.66	0.99
MUN,[5,9] mg/dL	4.42[a]	5.78[b]	8.64[c]	0.60	6.24	6.31	0.48	<0.01	0.92	0.17
MUN,[6] g	17.11[a]	32.32[b]	33.86[b]	4.58	30.19	25.33	3.66	0.02	0.34	0.37

[a–c]Within a row, means for nutritional treatment differ ($P \leq 0.05$).

[1]Nutritional treatments were RES (60% of control), CON (control; 100% requirements for gestating ewe lambs), and HIGH (140% of control).

[2]Selenium treatments were daily intake of organically bound Se, adequate Se (ASe; 9.5 μg/kg of BW) vs. high Se (HSe; 81.8 μg/kg of BW).

[3]Probability values for effects of nutrition (Nut), selenium (Se), and the interaction.

[4]Colostrum collected 3 h postpartum.

[5]Analysis conducted by Heart of America DHIA (Manhattan, KS).

[6]Total, g = weight of colostrum × [% item (butterfat, protein, lactose, solids not fat, MUN)/100)].

[7]SCC = somatic cell count.

[8]Total SCC = SCC, number of cells/mL × volume of colostrum, mL.

[9]MUN = milk urea nitrogen.

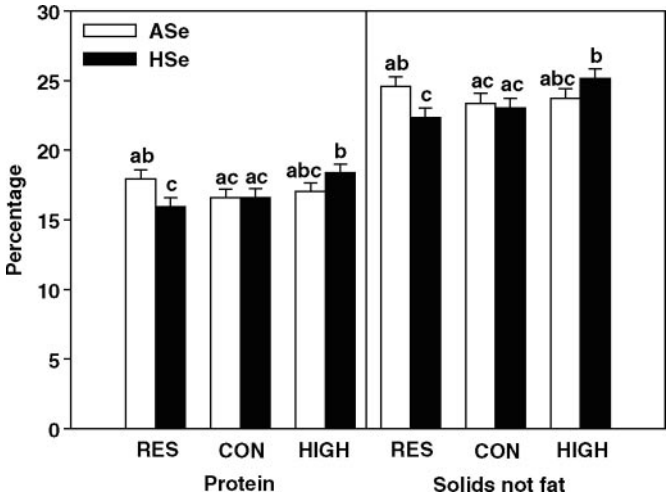

Figure 2. The effects of Se and nutritional level on protein and solids not fat percentages in colostrum of pregnant ewe lambs. Nutritional level × Se interaction ($P < 0.05$; [a–c]means ± SE within a measure differ). Nutritional treatments were RES (60% of control), CON (control; 100% requirements for gestating ewe lambs), and HIGH (140% of control). Selenium treatments were daily intake of organically bound Se, adequate Se (ASe; 9.5 μg/kg of BW) vs. high Se (HSe; 81.8 μg/kg of BW).

Although others have shown that Se supplementation decreased SCC in ewes (Morgante et al., 1999), we did not observe any alterations in SCC by maternal Se or nutritional intake.

The novelty of our experimental design allows for a clear evaluation of gestational nutrition on both maternal and neonatal outcomes. Lambs from under- and overnourished ewes may be less vigorous, and therefore, reductions in colostrum yield, along with a reduction in suckling vigor, would affect offspring health. In cattle, Hough et al. (1990) did not observe a decrease in colostrum IgG concentration in restricted dams; however, calves had decreased circulating IgG after suckling. This was attributed to decreased calf vigor at birth. If neonatal vigor is adequate, then health and survivability are due to the following: 1) the ability of the gastrointestinal tract of the neonate to adequately absorb IgG and other nutrients and 2) the ability of the mammary gland to provide the adequate nutrient supply. Studies examining the independent effects of maternal diet on growth and physiology of the mammary gland and offspring are limited, and data are needed to delineate gestation and lactational effects on offspring growth and health.

Slight modifications (i.e., either under- or over nutrition) in ewe intake from mid to late pregnancy can alter the colostrum yield and composition. Furthermore, the size of the mammary gland is not a good predictor of colostrum yield or quality, because mammary gland weight between control and overnourished ewe lambs was similar. Supplemental feed to enhance intake over NRC recommendations during mid to late gestation may be potentially detrimental to production in ewe lambs.

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